PATENT
Docket No.: 15270J-004765US
Client Ref. No.: 209-US-CIP8BC5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 9431
DALE B. SCHENK et al.	Examiner: Kimberly A. Ballard
Application No.: 09/724,288	Art Unit: 1649
Filed: November 28, 2000	DECLARATION UNDER 37 CFR § 1.131
For: PREVENTION AND TREATMENT OF AMYLOIDOGENIC DISEASE	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, Frederique Bard, Ted Yednock and Dale Schenk, the co-inventors of the above-captioned application state as follows:

1. We understand the above-captioned application has claims directed to a method of screening an antibody for activity in clearing an amyloid deposit of Aβ, comprising combining the amyloid deposit, the antibody, and microglial cells bearing Fc receptors in a medium in vitro; and by a series of measurements monitoring a reduction in the amount

of the amyloid deposit remaining in the medium, the reduction in the amount of the amyloid deposit indicating the antibody has clearing activity against the amyloid-deposit.

2. We have reviewed Brazil et al, J. Biol. Chem. 275, 16941-16947 (2000). We have been told to assume for purposes of this declaration only that Brazil et al. has a publication date of March 23, 2000. Brazil et al. discuss an experiment in which aggregated Aβ was contacted with an antibody to Aβ and then with microglial cells in comparison with a control in which microglial cells were contacted with Aβ alone. It was reported that the antibody increased internalization of the aggregates of Aβ by the microglia by about 1.5 fold.

3. We had completed our invention as described above, and in particular so much of our invention as might be alleged to be disclosed or suggested by Brazil et al. before the publication of publication date of Brazil et al.

4. It is our practice to keep a contemporaneous and chronological account of work in bound laboratory notebooks. We attach true copies of pages from Notebook 2298 of Frederique Bard. The work described in these pages was performed in the United States. Dates on which work was performed and witnessed have been redacted. The following paragraph summarizes work performed by one or more of us or a technician acting under the supervision of one or more us in the attached notebook pages.

5. The experiment described in the attached notebook pages was designed to test whether antibody 10D5 could clear amyloid deposits of Aβ obtained from a PDAPP mouse. Cryostat sections of a PDAPP mouse brain were collected onto poly D-lysine coated coverslips and coverslips were placed into a 24-well plate. Sections were either pretreated with 1% triton/PBS for 45 min or not, then washed, and incubated in assay medium overnight at 37°C. Sections were then washed with assay medium, and one PDAPP section was pre-incubated for 45 min with the monoclonal antibody 10D5 at a final

concentration of 10 ug/ml,. 0.67 x 10^6 mouse microglial cells per well were then added and the co-culture (microglia and cryostat section) incubated for 24hr at 37°C. At the end of the incubation, the co-cultures were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton-X100, and stained with biotinylated 3D6 followed by a streptavidin / PE conjugate. The nuclei of microglial cells were visualized by a nuclear stain (DAPI). 6. The micrographs on p. 11, p.12, and p.13 show pictures that illustrate the results. The picture on the left on p.11 shows that there was no punctate staining of Aβ on triton-treated sections co-cultured with microglial cells but without 10D5 antibody. The picture on the right and on p.12 show punctate staining of Aβ on a section that was treated with 10D5. The photograph on p.13 shows that the punctate staining of Aβ is associated with the microglial cells identified by the DAPI staining, demonstrating internalized Aβ within the cells. Thus upon treatment of the cryostat sections with 10D5, the microglial cells were able to phagocytose Aβ plaques.

7. As a result of this experiment, we knew the 10D5 antibody had activity in clearing an amyloid deposit of Aβ.

8. We further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: _____ Dated: Dec 19, 2006
Frederique Bard

Signed: _____ Dated: Dec 19, 2006
Ted Yednock

Signed: _____ Dated: Dec 19, 2006
Dale Schenk

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